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EXHIBIT B

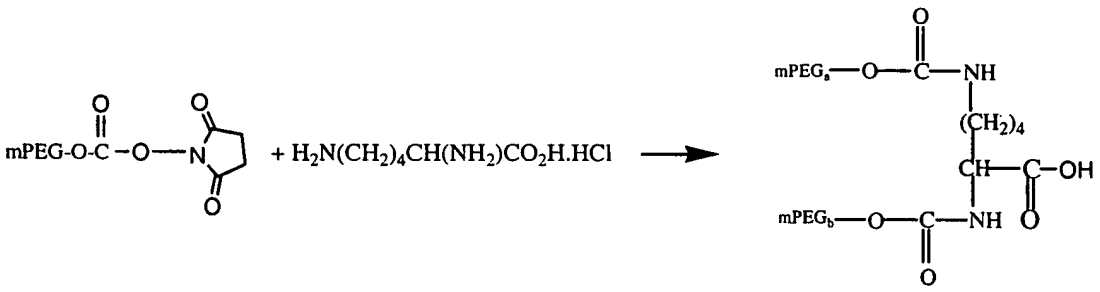
Preparation of PEG-2 Lysine by an Alternative Route and Purification of the Crude Mixture by Recrystallization

Experiment 2.

I. Purpose: To examine the effectiveness of recrystallization in removing polymeric impurities arising during the synthesis of a branched PEG polymer, mPEG-disubstituted lysine, "mPEG-2-LYS", was prepared in accordance with the teachings set forth in the instant patent application, U.S. Serial No. 10/119,546.

II. Materials: The materials used in the synthesis of mPEG-disubstituted lysine, MW 40,000 daltons (20 kD for each polymer arm) can be found in Batch Record # PEG2NHS40KP-028.

III. Synthesis Method: Preparation of Crude mPEG-2-LYS.



Purified water (13,000 mL) was added to a cylindrical reactor with stirring, followed by addition of 81 g of boric acid. The pH of the resulting solution was adjusted to approximately 8 by addition of a 200 mL of a 1.0 N solution of sodium hydroxide. To this solution was added a solution of lysine monohydrochloride (10.1 g) in water (50 mL). Methoxy-PEG-Succinimidyl Carbonate, 20 kD (mPEG-SC), 2469 g, was then quickly added to the solution of lysine monohydrochloride, followed by dropwise addition of a 1.0 N sodium hydroxide solution to maintain the pH at approximately 8. The resulting solution was then stirred for 2.5 hours at 19-30° while maintaining the pH at approximately 8. The pH of the aqueous reaction mixture was then adjusted to approximately 3.0 by addition of acid.

220 mL of the above crude reaction mixture were then withdrawn for further purification. To this reaction mixture was added 30 g of sodium chloride, followed by extraction of the aqueous solution with dichloromethane (two extractions were performed, each with 200 mL solvent). The organic extracts were then combined and dried over approximately 80 g of anhydrous sodium sulfate. The organic mixture was stirred for approximately 1 hour, and the solvent removed by rotary evaporation to yield a viscous syrup referred to herein as crude mPEG-2-LYS.

From this viscous syrup was withdrawn approximately 3 g of material, which was then further dried overnight under high vacuum to provide dried, recovered crude

mPEG-2-LYS (1.8 g) for use as a control for further comparison, referred to herein as "**Sample 1**".

IV. Purification Method(s).

Crude mPEG-2-LYS as described above was then submitted to two different methods of purification and subsequent analyses. In the first method, crude mPEG-2-LYS was purified by recrystallization, the method of purification described in U.S. Patent No. 5,643,575. In the second method, crude mPEG-2 LYS was purified by ion exchange chromatography.

A. Recrystallization. Isopropyl alcohol (200 mL) was added to the crude mPEG-2-LYS. The resulting mixture was then warmed to approximately 60°C in a hot water bath until all of the solids dissolved. The solution was then slowly cooled to room temperature, during which time a white precipitate formed. The solids were recovered by filtration, followed by washing with 100 mL of isopropyl alcohol followed by 50 mL of diethyl ether. The recrystallized product was then dried under high vacuum (2.2 g, **Sample 2**).

B. Chromatography. Crude mPEG-2-LYS was purified by ion exchange chromatography using a DEAE-sepharose column essentially following the procedure provided on page 27 of the Applicant's patent application, U.S. Serial No. 10/119,546. This chromatographically-purified sample is referred to herein as **Sample 3**.

V. Results:

The above samples were analyzed by two different gel permeation chromatography (GPC) methods to evaluate the purity of the isolated, purified mPEG-2-LYS products.

A. Analysis.

GPC Method 1 employed an Ultrahydrogel column and sodium phosphate buffer as the mobile phase; **GPC Method 2** employed a Mixed-D column and dimethylformamide as the mobile phase. Details of the GPC parameters employed for the two different analytical methods were as follows.

Table 1. GPC Method 1 - Operating Parameters and Elution Times

HPLC SYSTEM	HP 1100 w/ HP 1047A RI Detector
GPC COLUMN	Ultrahydrogel 250™, Waters
MOBILE PHASE	5 mM sodium phosphate buffer, pH 7.2
SAMPLE CONCENTRATION	20 mg/1 mL 5 mM phosphate buffer
INJECTION VOLUME	5 microliters
COLUMN CHAMBER TEMPERATURE	75° C
FLOW RATE	0.5 mL/minute
PEAK 1: PEG-2-LYS, PEG-3	elution time: 12.5 minutes
PEAK 2: MPEG, 20 K AND MPEG1-LYS	elution time: 13.6 minutes

Structures corresponding to various PEGs referred to herein are provided in Table 5.

Table 2. GPC Method 2 - Operating Parameters and Elution Times

HPLC SYSTEM	Waters System 2690 with an RI Detector
GPC COLUMN	Mixed-D columns, dual, in series, Polymer Laboratories
MOBILE PHASE	anhydrous N,N-dimethylformamide (DMF)
SAMPLE CONCENTRATION	10 mg/2 mL anhydrous DMF
INJECTION VOLUME	30 microliters
COLUMN CHAMBER TEMPERATURE	70° C
FLOW RATE	0.5 mL/minute
PEAK 1: PEG-3	15.2 minutes
PEAK 2: PEG-2-LYS	17.1 minutes
PEAK 3: MPEG, 20 K AND PEG-1-LYS	23.7 minutes

Samples Analyzed: The following samples were analyzed.

Table 3.

Sample I.D.	Identity
Sample 1	Control: Crude mPEG-2-LYS prior to purification by any method
Sample 2	Recrystallized mPEG-2-LYS
Sample 3	Chromatographed mPEG-2-LYS
Sample 4	mPEG, 20 kD (used in prep of mPEG-2-LYS)

B. Analytical Results.

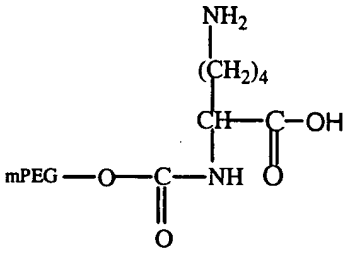
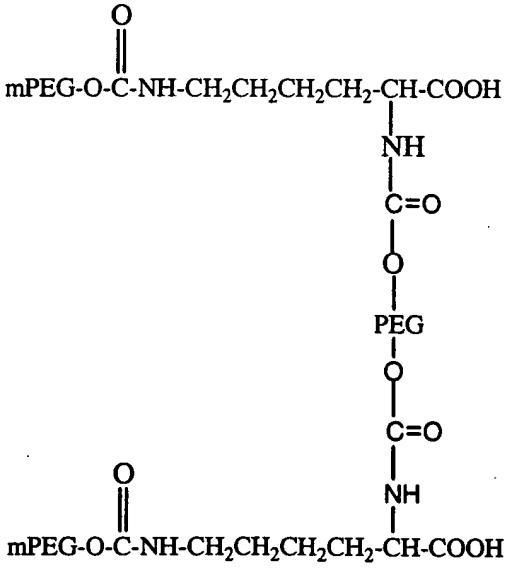
Table 4. Summary of Results

Each column below summarizes the relative amount of PEG compound identified in column one, as a percent of total, detected in the sample by each of the 2 methods employed. Specifically, shown in each column is the amount of PEG compound detected by GPC Method 1/amount of PEG compound detected by GPC Method 2.

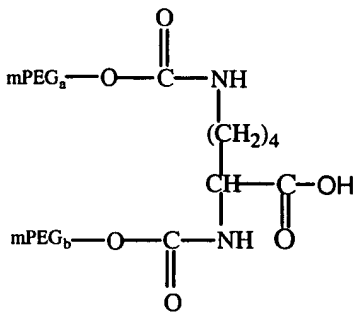
PEAK IDENTITY	SAMPLE 1 GPC 1*/GPC 2	SAMPLE 2 GPC 1*/GPC 2	SAMPLE 3 GPC 1*/GPC 2
mPEG-2-LYS	79.5 % / 65.3 %	**79.8 % / 67.4 %**	100 % / 100 %
mPEG, mPEG-1-LYS	20.5 % / 23.7 %	**20.2 %/ 20.2 %**	0 % / 0 %
PEG-3	0 % /12.3 %	**0 % / 12.4 %**	0 % / 0 %

*Using GPC Method 1, PEG 3 is inseparable from the product, mPEG-2 LYS, and falls under the reported mPEG-2-LYS peak. Separation/detection of PEG-3 is achieved, however, using GPC Method 2.

Table 5. Relevant Structures.

Abbreviation	Structure
mPEG	$CH_3O\text{-}(CH_2CH_2O)_n\text{-}H$
Lysine (LYS)	$H_2N(CH_2)_4CH(NH_2)CO_2H$
PEG-1-LYS	
PEG3	

PEG-2-LYS



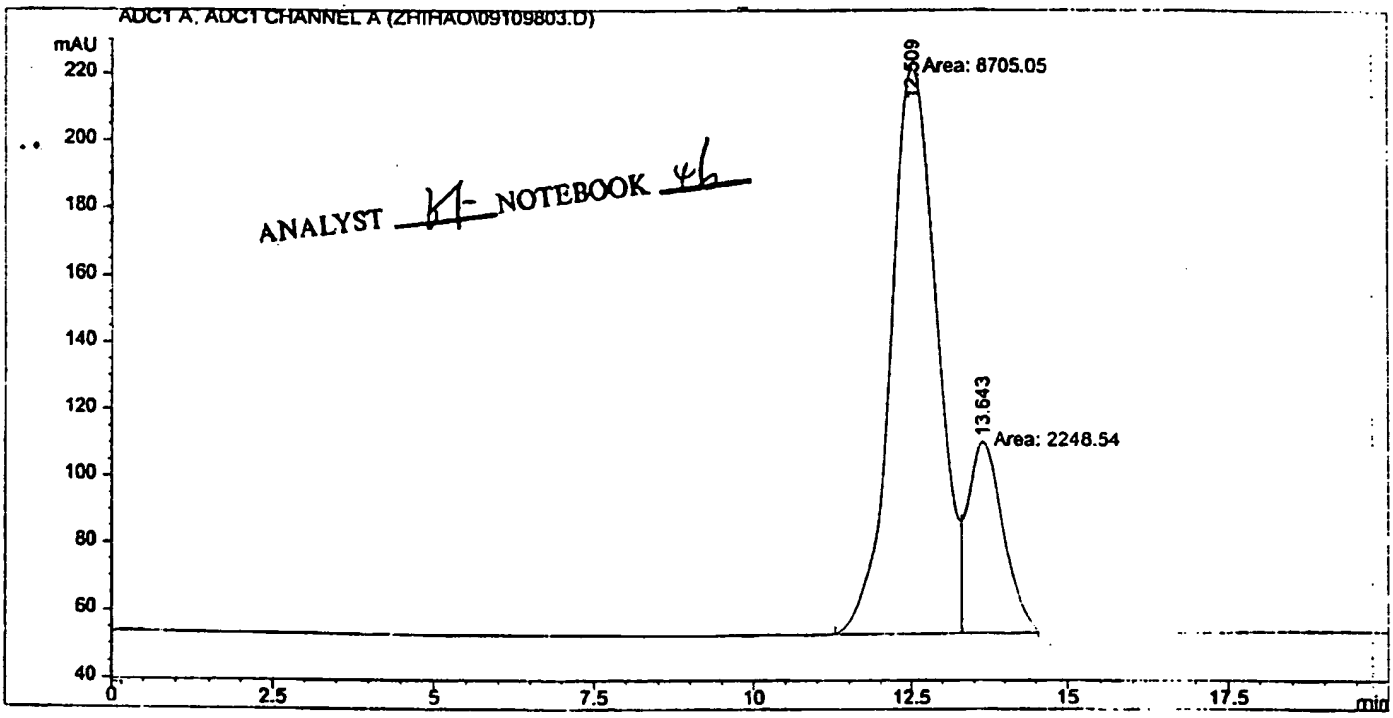
VI. Conclusion:

The analytical results for Sample 2, recrystallized mPEG-2-LYS, demonstrate that the material, Sample 1. Specifically, sample 2 contains approximately 20% combined mPEG and mPEG-1-LYS and 12% PEG-3; in total, Sample 2 contains approximately 30% polymeric impurities. Recrystallization of crude PEG-2-LYS resulted in essentially no improvement in purity and is an ineffective method for removing polymer impurities resulting from the preparation of branched polymers such as mPEG-2-LYS.

Purification of crude mPEG-2-LYS by ion exchange chromatography, Sample 3, provides a purified branched polymer product that lacks detectable quantities of polymeric impurities such as mPEG, mPEG-1-LYS, and PEG-3, as assessed by GPC.

VII. FIGURE LEGEND.

FIG. 1. GPC Method 1. Trace of Sample 1.
FIG. 2. GPC Method 1. Trace of Sample 2.
FIG. 3. GPC Method 1. Trace of Sample 3.
FIG. 4. GPC Method 2. Trace of Sample 1.
FIG. 5. GPC Method 2. Trace of Sample 2.
FIG. 6. GPC Method 2. Trace of Sample 3.
FIG. 7. GPC Method 2. Trace of Sample 4.



ANALYST _____ NOTEBOOK _____

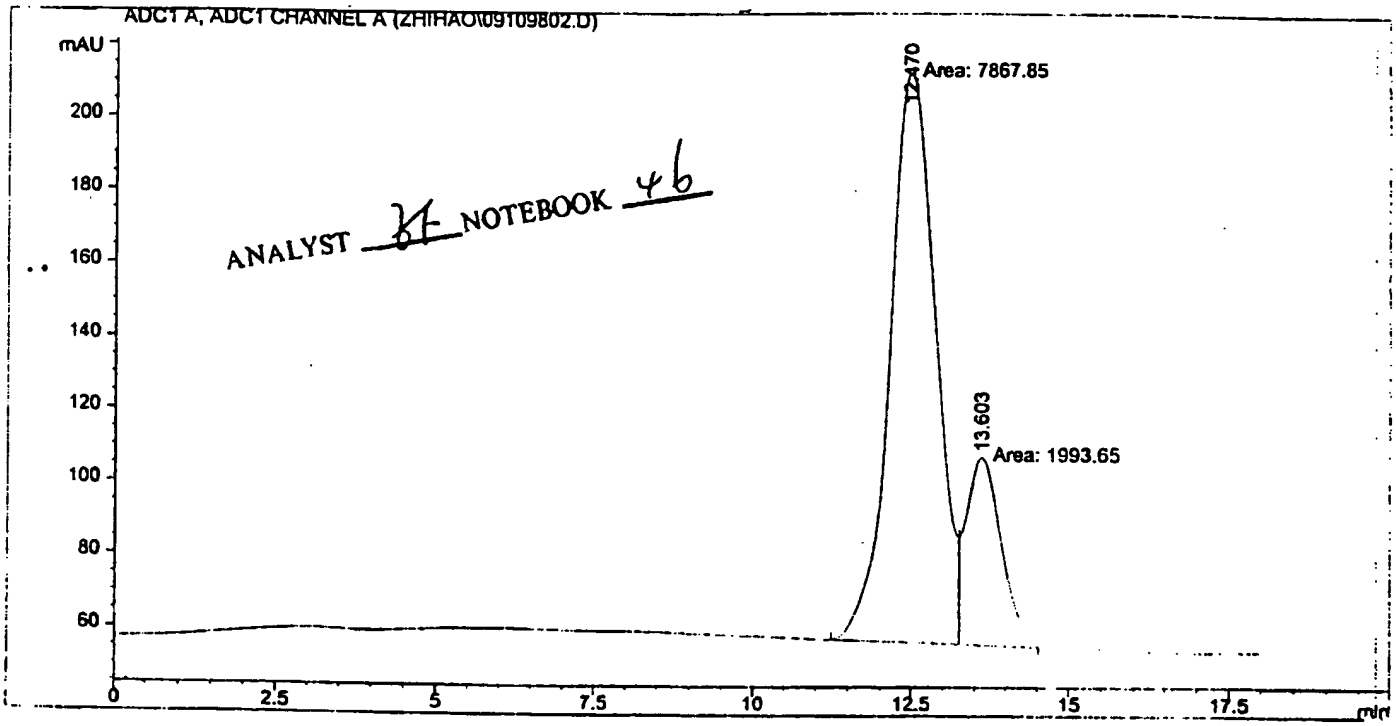
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ADC1 A, ADC1 CHANNEL A

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.509	MF	0.8624	8705.04883	168.23587	79.4721
2	13.643	FM	0.6569	2248.54443	57.04516	20.5279
Totals :				1.09536e4	225.28103	

FIG. 1



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Area Percent Report

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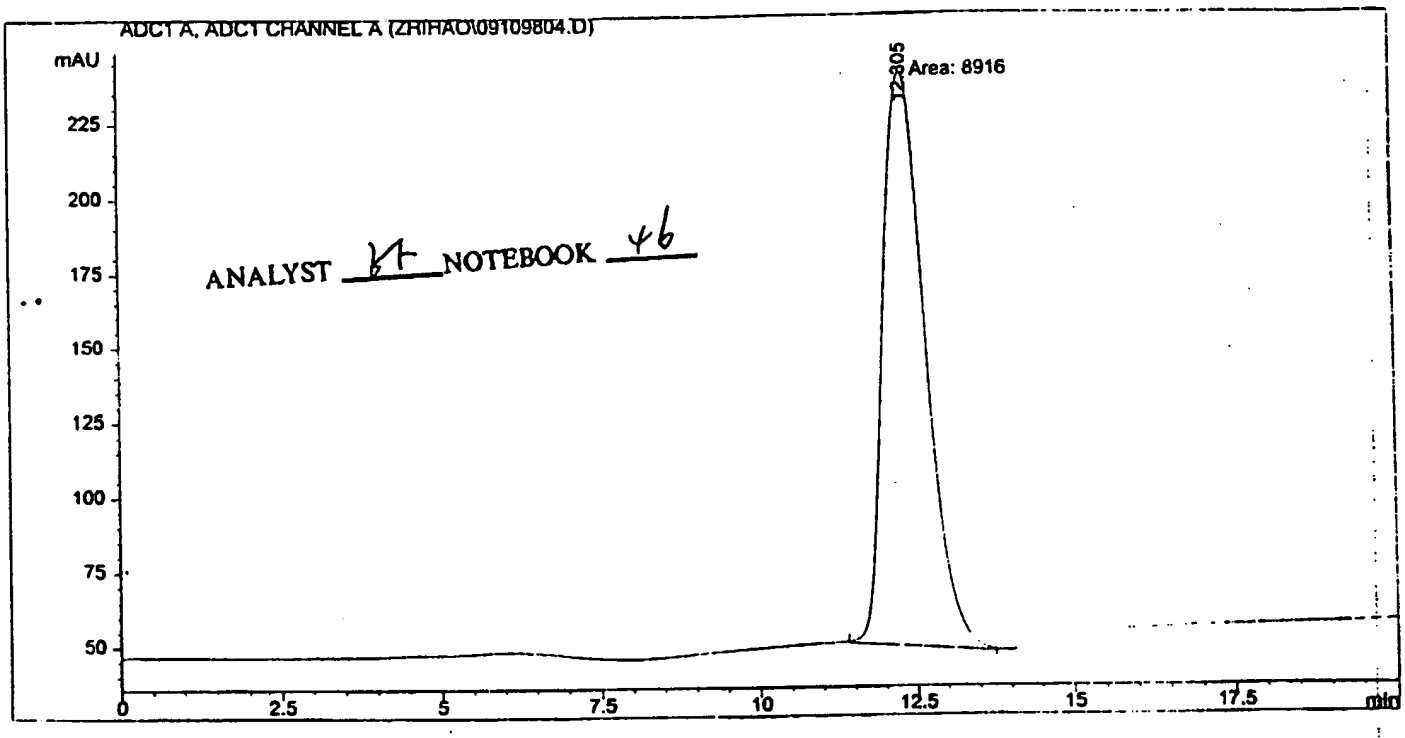
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ADC1 A, ADC1 CHANNEL A

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.470	MF	0.8398	7867.85400	156.14349	79.7835
2	13.603	FM	0.6436	1993.64697	51.62801	20.2165
Totals :				9861.50098	207.77151	

FIG. 2



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Area Percent Report

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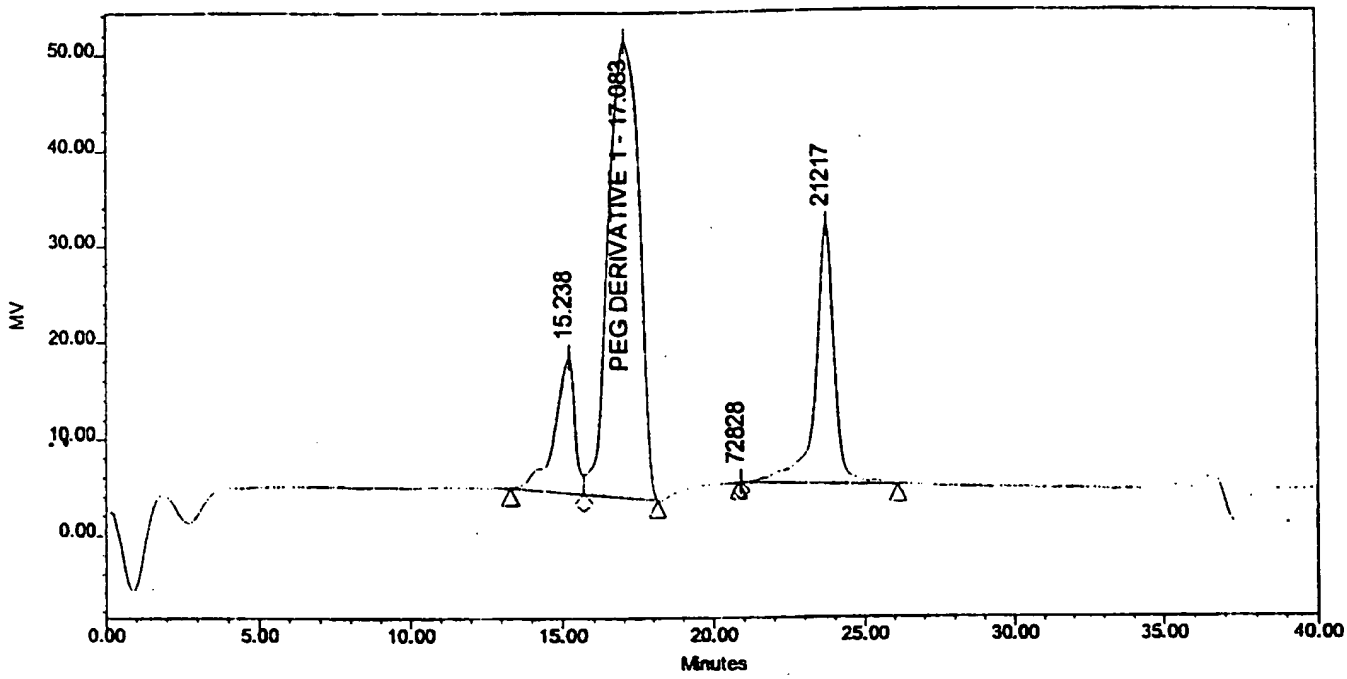
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: ADC1 A, ADC1 CHANNEL A

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.305	MM	0.7793	8916.00000	190.68658	100.0000

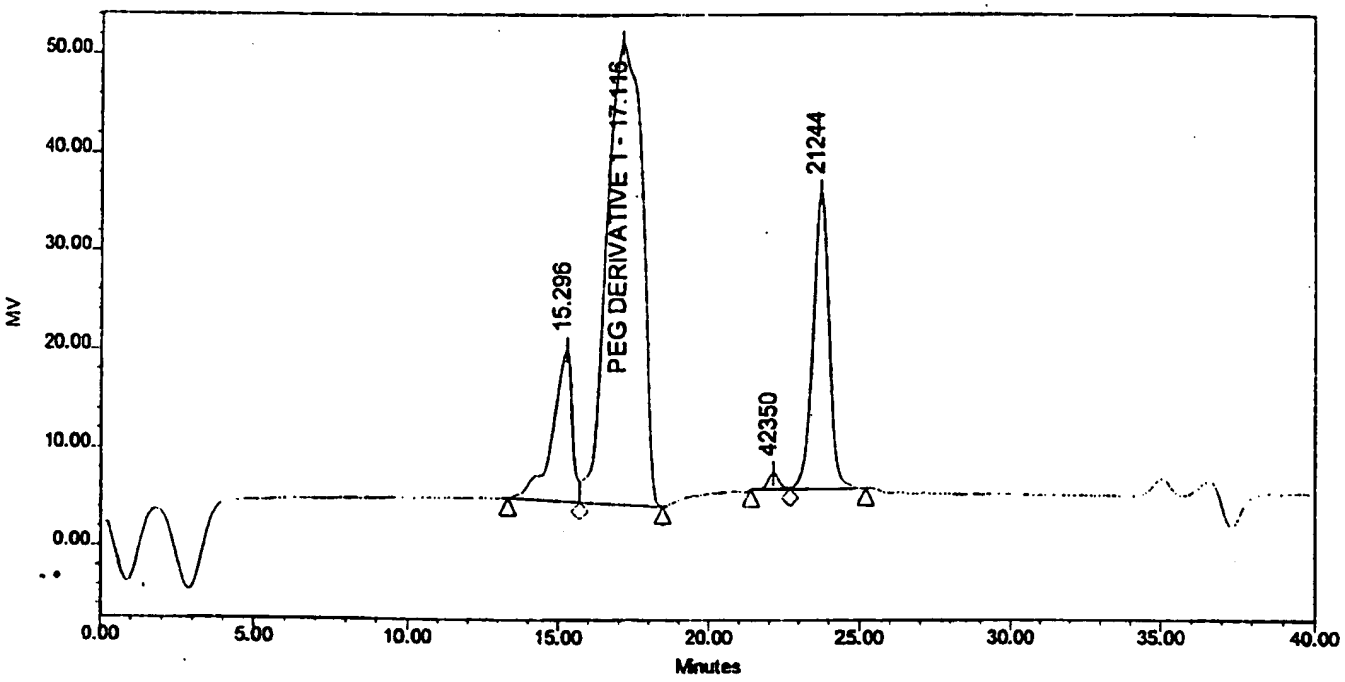
FIG. 3



GPC Results

	Dist Name	Area	% Area	Retention Time	Mn	Mw	MP	Mz	Mz+1	Polydispersity
1		646932	12.30	15.238						
2		3436504	65.32	17.083	303999	304840		305646	306417	1.002767
3		148	0.00	20.917			72828			
4		1177111	22.38	23.731	21668	22950	21217	24729	27396	1.059172

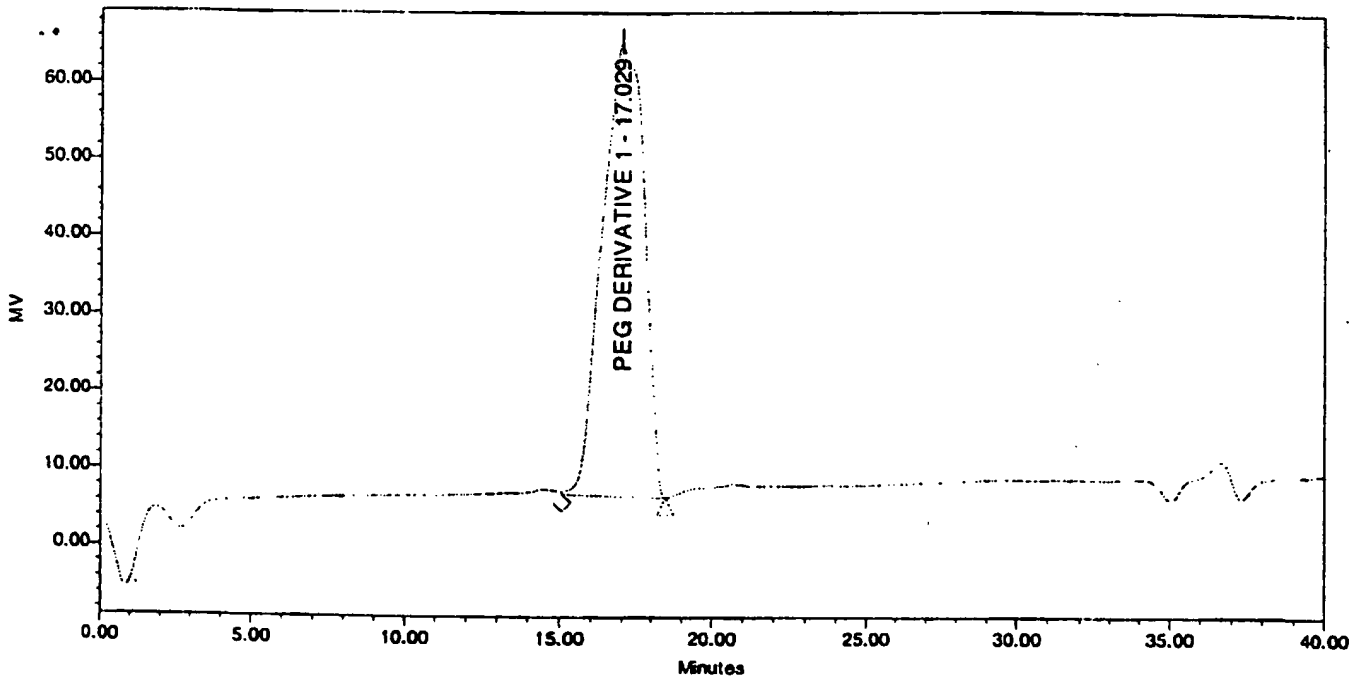
FIG. 4



GPC Results

	Dist Name	Area	% Area	Retention Time	Mn	Mw	MP	Mz	Mz+1	Polydispersity
1		701330	12.40	15.296						
2		3810383	67.38	17.116	294315	295782		297185	298524	1.004985
3		45628	0.81	22.154			42350			
4		1097328	19.41	23.728	21080	21377	21244	21676	21979	1.014108

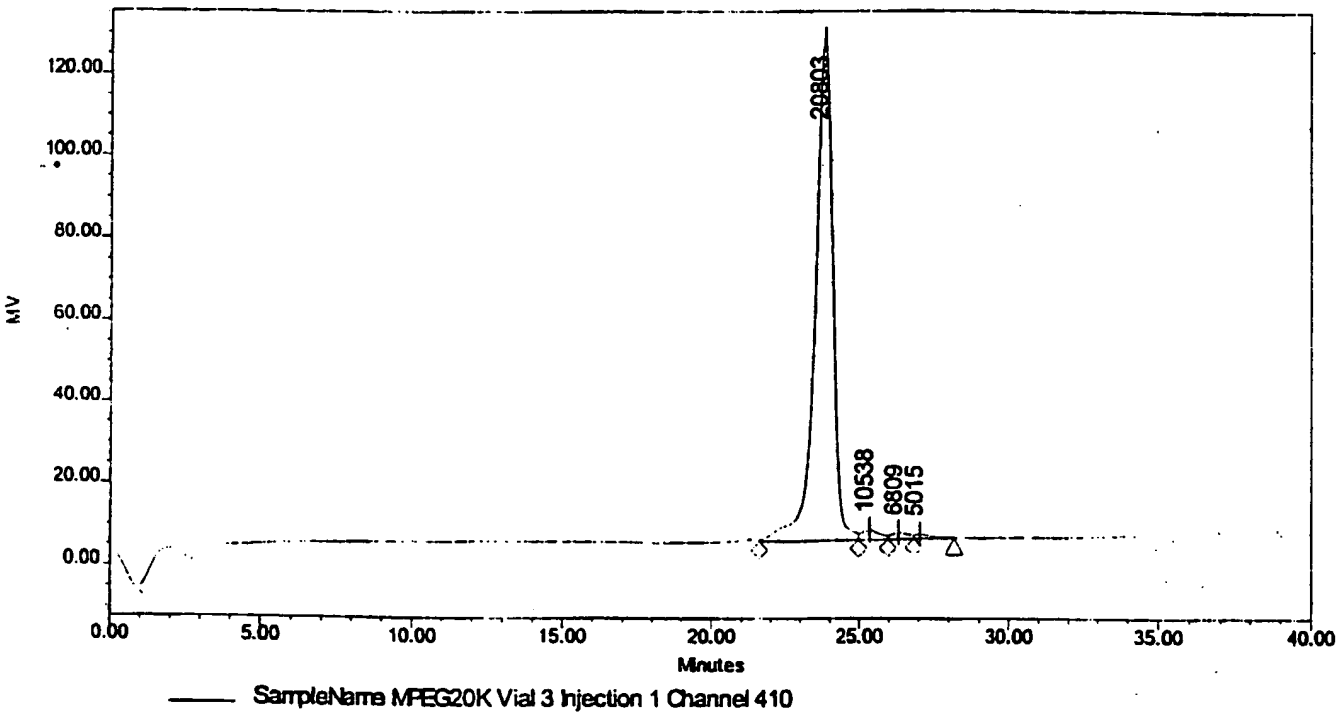
FIG. 5



GPC Results

	Dist Name	Area	% Area	Retention Time	Mn	Mw	MP	Mz	Mz+1	Poly dispersity
1		5621419	100.00	17.029	291157	293051		294849	296550	1.006502
2				*24.109*						

FIG. 6



GPC Results

	Dist Name	Area	% Area	Retention Time	Mn	Mw	MP	Mz	Mz+1	Polydispersity
1				17.566						
2		5060480	96.15	23.786	21216	21875	20803	22769	24047	1.031059
3		99136	1.88	25.338			10538			
4		63703	1.21	26.335			6809			
5		39600	0.75	27.033			5015			

FIG. 7

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